A PROPOSED EXPLANATION FOR THE ORIGIN OF COLCHICINE-INDUCED DIPLOID MUTANTS IN SORGHUM [1]

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COLCHICINE, AN ALKALOID extracted from plants of *Colchicum* species, especially *C. autumnale* L., has long been used for medicinal purposes. In tissues of animals which have been exposed to the drug, mitoses are arrested with the result that some cells are produced with a doubled number of chromosomes (Eigsti & Dustin, 1955, Chap. 1). Botanists realized that such tetraploid cells in plant tissues might give rise to tetraploid shoots and plants, and this proved to be the case (Blakeslee, 1937; Nebel & Ruttle, 1938). In many plant species, colchicine treatment has been used successfully to produce polyploids for cytogenetic research and for breeding programs (Eigsti & Dustin, 1955, Chap. 11).

In the breeding program at South Dakota State College, colchicine treatment of *Sorghum* was found to produce, in addition to polyploids, diploid mutant plants changed for many characters. When the mutants were selfed, some produced uniform progenies and continued to breed true in succeeding generations; others produced progenies which segregated for many characters (Franzke & Ross, 1952).

'Experimental 3' (FIG. 1), which has given rise to many colchicine-induced diploid mutants, has been studied more extensively than other lines. It was developed from crosses involving three sorghum lines: 'Day Milo', a grain sorghum (*Sorghum vulgare* Pers. var. *subglabrescens* (Steud.) A. F. Hill); 'Black Amber Cane', a sorgo (*S. vulgare* var. *saccharatum* (L.) Boerl.); and Sudan Grass, a grass sorghum (*S. sudanense* (Piper) Stapf). Two crosses, 'Day Milo', with 'Black Amber Cane' and 'Day Milo' with Sudan Grass, were made in 1932. An early dwarf grain type was obtained from each of these crosses by repeated selfing and selection. These two types were crossed together in 1939, and from the progeny of this cross, 'Experimental 3' was developed by continued selfing and selection. It had been through eight generations of selfing and was true-breeding when the first mutants were obtained in 1948. After more than 20 generations of selfing, it is still apparently the same true-breeding

[1] The authors are sincerely grateful to Dr. Richard A. Howard, Director of the Arnold Arboretum, for making possible the continuation of the work, and to members of the Arboretum staff for all their assistance. We also thank those who gave their time to review the manuscript: Dr. G. Lefevre, Jr., Dr. P. C. Mangelsdorf, Dr. M. R. Morris, Mr. J. Munoz, Dr. L. I. Nevling, Jr., Mr. P. Nordquist, Dr. R. C. Rollins, Dr. B. G. Schubert, Dr. O. T. Solbrig and Dr. O. J. Webster. Their many valuable suggestions are greatly appreciated.

The research on which the paper is based was supported in part by the National Science Foundation and the National Institutes of Health.

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line and continues to give rise to colchicine-induced diploid mutants some of which resemble one of the original parents. For example, there are mutants with slender stems, narrow leaves and open panicles (Class I, Franzke & Sanders, in press) similar to Sudan Grass. Except where otherwise specified, the following discussion relates to sorghum line 'Experimental 3' and its derivatives.

This paper brings together the results of research on colchicine-induced diploid mutants in *Sorghum* and, in the light of available information, proposes a new hypothesis to explain their origin. The significance of the hypothesis, if proved valid, is also considered.

COLCHICINE TREATMENT OF SORGHUM LINE 'EXPERIMENTAL 3'

Sorghum plants are treated with colchicine at the seedling stage. Shortly after germination, a mixture of 0.5 per cent colchicine in lanolin, heated to the melting point, is applied to cover the coleoptile. Treated seedlings have been planted in various media and kept under various conditions in greenhouse, laboratory, or rooms with controlled light and temperature. Both polyploids and diploid mutants have been obtained in the treated generation. There are indications that environmental conditions may influence the outcome of treatment.

Polyploids

Experimentally produced polyploids of 'Experimental 3' are, for the most part, tetraploid plants which exhibit the same qualitative characters as diploid plants, but are shorter and stockier with broader stems, heavier leaves, larger glumes and grain, and have a high degree of sterility. These characteristics are similar to those of polyploids in many other plant species. Self-progenies of these polyploids frequently include both diploid and polyploid plants, indicating that the treated plants were chimeras. The incidence of induction of polyploids, which has been as high as four out of 15 surviving treated seedlings, appears to be increased by conditions which favor the growth of treated seedlings (Franzke *et al.*, 1960).

Diploid Mutants

Diploid mutants exhibit qualitative and quantitative characters which are radically different from those of 'Experimental 3' plants. Yet, the changes have been brought about by colchicine after the germination of an 'Experimental 3' seed. Uniform self-progenies of two diploid mutants are shown in FIGS. 2 and 3; each plant repeats the characters of the original mutant. A segregating self-progeny of a nontrue-breeding mutant is shown in FIG. 4. In progenies of this kind, most characters may segregate, or some may segregate while others do not (Sanders *et al.*, 1962). Thus, colchicine-induced diploid mutants may be true-breeding, nontrue-breeding, or intermediate between the two conditions. The incidence of induction of diploid mutants, especially true-breeding ones, which has been as high as


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four out of nine surviving treated seedlings, appears to be increased by conditions which retard the growth of treated seedlings (Franzke *et al.*, 1960). The largest proportion of mutants among surviving treated plants was obtained under conditions which almost eliminated survival. Maintaining treated seedlings at a lower temperature (68° F.) increased survival while still permitting induction of mutants (Sanders *et al.*, 1960).

Some types of true-breeding mutants were found to recur thereby forming classes of almost identical mutant lines (Franzke & Sanders, in press). Some classes included as many as ten lines, most of them obtained within a five-year period of experimentation. Three lines from one class were intercrossed in all possible combinations and were each crossed to 'Experimental 3'. The results indicated that these three mutant lines were genotypically as well as phenotypically alike (Chen *et al.*, 1961).

The original hypothesis proposed for the origin of the diploid mutants was "that such variant plants could originate through reductional grouping of the somatic chromosomes so that a concentration of chromosomes containing gene blocks originating from one of the ancestors of the polyploid species might occur in one cell" (Franzke & Ross, 1952).

When no irregularities were detected in chromosome-pairing relationships at pachytene in mutants or in F_1 plants from crosses between mutants and other lines including parental ones, the original proposal was discarded. It was then concluded "that no detectable rearrangement of chromatin occurred," and "that the colchicine-induced variants have resulted from gene mutation or cryptic structural changes in the chromatin" (Harpstead *et al.*, 1954). Genetic studies of one of the mutants led to an estimate that "at least 12 (in all probability more) immediate mutations for the characters studied" had occurred (Foster *et al.*, 1961).

Current information, however, suggests that the origin of the mutants is more similar to the first proposal since the variations in them resemble major chromosome changes more than individual gene changes, despite the lack of confirming cytological evidence.

PRESENT HYPOTHESIS FOR THE ORIGIN OF DIPLOID MUTANTS

It is now proposed that colchicine-induced diploid mutants arise from the substitution of chromosomes of similar phylogenetic origin (analogous chromosomes), and that the substitutions have not been detected cytologically because there is a tendency for bivalent rather than multivalent

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FIGS. 1–4. Progeny rows of sorghum line 'Experimental 3' and of three diploid mutants obtained from 'Experimental 3' after colchicine treatment. The yardstick indicates height. FIG. 1. 'Experimental 3'. FIG. 2. True-breeding mutant. Class IV (Franzke & Sanders, in press). FIG. 3. True-breeding mutant. Class VB (Franzke & Sanders, in press). FIG. 4. Nontrue-breeding mutant; leaf width, head type, height, and maturity can be seen to segregate. Height differences are indicated by the bags which cover the main heads. Maturity differences are indicated by late unbagged plants, i.e., the first in the row, and early plants with well developed tillers which account for many of the unbagged heads.

pairing to occur in sorghums with $2n = 20$, and because pairing may occur between analogous chromosomes. Information from the literature, as well as research on the mutants, lends support to this view.

Major Chromosome Changes

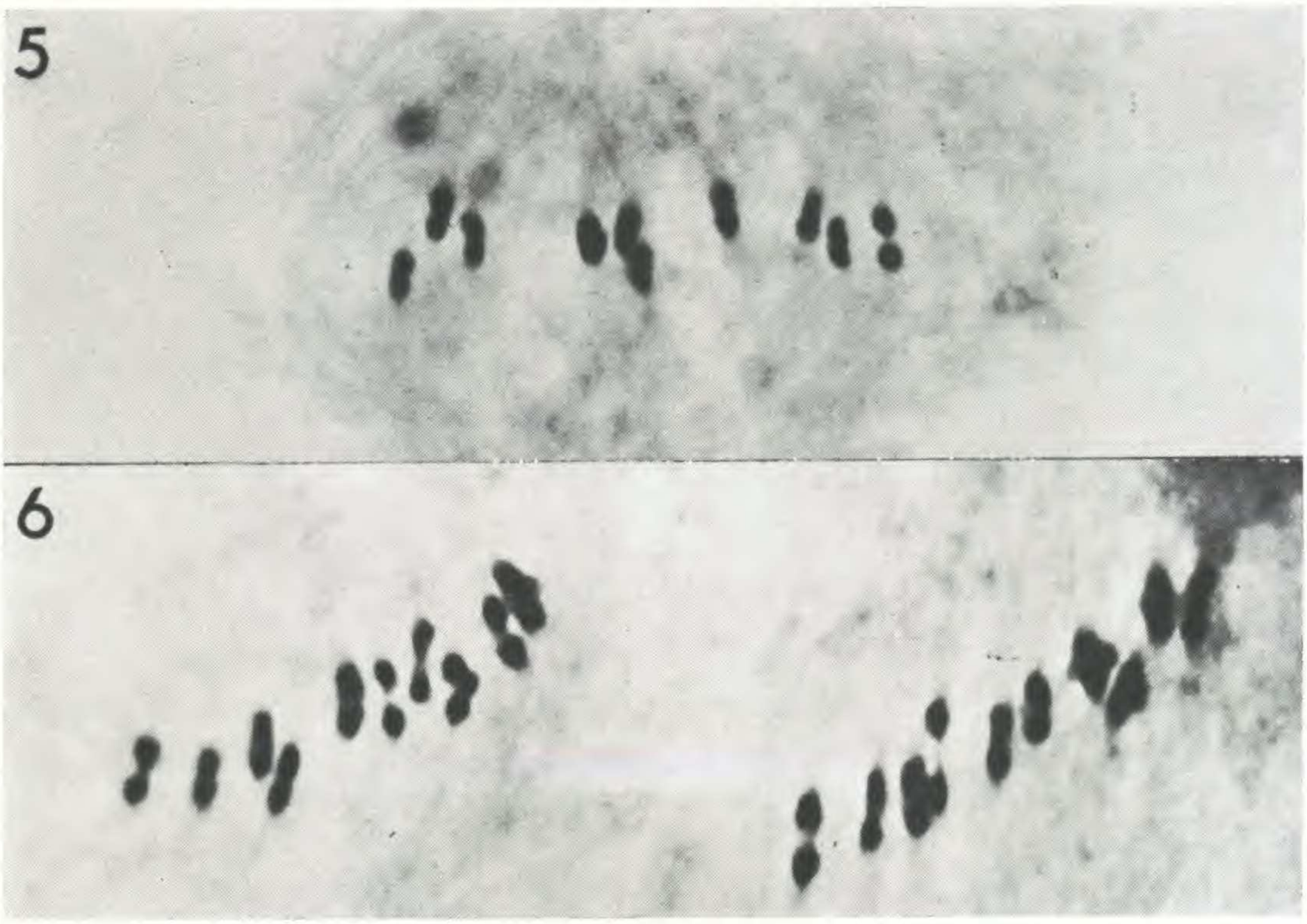
The phenotypic changes in the colchicine-induced diploid mutants appear to come about through major chromosome rearrangements since a large number of qualitative and quantitative characters are involved. Moreover, they are similar to natural variations found in sorghum varieties, and they include types which recur.

Similarly, complex mutants in other species have been shown to result from the addition or loss of entire chromosomes, i.e., in *Datura stramonium* L. (Blakeslee & Belling, 1924) and in *Lycopersicon esculentum* Mill. (Rick & Barton, 1954). In these species, the same complex mutants recurred and were shown to result from the addition or loss of the same chromosomes, either original chromosomes as in primary trisomics or monosomics, or isochromosomes as in secondary trisomics. Vasek (1956) working with *Clarkia unguiculata* Lindl., a highly variable outcrossed species, concluded that aneuploid types probably have characteristic phenotypes only in highly homozygous diploid species. Otherwise effects of aberrant chromosomes may be either obscured by variability among the diploid plants, as in *Zea Mays* L. (McClintock, 1929), or overshadowed by the effects of duplicated genes in various genomes, as in hexaploid *Triticum aestivum* L. (Sears, 1944, as *T. vulgare* Vill.).

Plants with extra chromosomes have been found in *Sorghum* (Endrizzi & Morgan, 1955; Hadley & Mahan, 1956; Price & Ross, 1957), but not in sufficient numbers in the same true-breeding line to establish whether or not the presence of a particular extra chromosome produces a particular phenotype. Since 'Experimental 3' is true-breeding and has a chromosome number of $2n = 20$, chromosome mutants in this line might be expected to be phenotypically distinct. If the distinct mutant complexes depend on the distribution of whole chromosomes, they would be likely to recur since the haploid chromosome number in *S. vulgare*, ten, is relatively small.

Substitution of Whole Chromosomes

The mechanism for chromosome changes would have to be substitution since there is no change in chromosome number in the mutants. However, since substitution has not been detected cytologically, there would have to be additional factors which prevent the expected changes in chromosome pairing relationships. More detailed studies may reveal direct cytological evidence for chromosome substitution. Irregularities in the structure of some bivalents at metaphase in a nontrue-breeding mutant (FIGS. 5 and 6) were originally disregarded as possible artifacts. However, their repetition from cell to cell suggests that the ten bivalents in the mutant may not be the same ten bivalents as in 'Experimental 3'. It should be possible to interpret such apparent differences by studying earlier meiotic stages.



FIGS. 5, 6. Sorghum chromosomes at metaphase I in pollen mother cells. FIG. 5. 'Experimental 3'. FIG. 6. Nontrue-breeding mutant G (Sanders *et al.*, 1962). Cells of the mutant have two relatively large irregular bivalents in addition to a large bivalent similar to the one in 'Experimental 3'.

Three Requisite Assumptions

ANALOGOUS CHROMOSOMES. The assumption that the haploid number of ten in *Sorghum vulgare* includes analogous chromosomes which can be substituted one for another without leading to inviability of the plant is basic. This assumption seems warranted as species of *Sorghum* with a haploid number of five are known, although not in the subgenus *Sorghum* [3] which includes *S. vulgare*.

Several investigators have concluded that the 20-chromosome diploid sorghums are of polyploid origin. Huskins and Smith (1934) based their conclusion primarily on the sporadic multivalents found in all species with $2n = 20$ studied, and on the frequent formation of multivalents in an asynaptic form of 'Dakota Amber Sorgo' in which normal pairing relationships were disrupted. Garber (1950) and Celarier (1958a), after examining the cytotaxonomic characteristics of the species of *Sorghum*, concluded that the "Eu-sorghums" are allopolyploids, i.e., *S. vulgare* with $2n = 20$ is an allotetraploid. Damon (1962) also considered that cultivated sorghums with $2n = 20$ are allotetraploids and suggested that if they have two genomes with slight homologies between corresponding chromosome pairs, the "secondary associations" frequently reported for *Sorghum* chromosomes could account for the inheritance of blocks of

[3] This subgenus should be called *Sorghum* rather than *Eu-sorghum* since it includes the type species (Art. 22, Int. Code Bot. Nomencl. 1961).

morphological characters frequently found following hybridization. He further pointed out that although Venkateswarlu and Reddi (1956) in their study of pachytene chromosomes of *Sorghum vulgare* var. *subglabrescens* (as *S. subglabrescens* (Steud.) Schwein. & Aschers.) identified ten distinct chromosome types, it seems feasible from their figures to arrange them as five pairs, the members of each pair differing primarily in arm length. A study of the paper itself reveals that, disregarding arm length, the chromosomes can be matched fairly well on the basis of the morphology of the heavily staining regions on either side of each centromere. The chromosomes have been numbered from 1 to 10 in order of decreasing length. The most probable pairs would appear to be: 1 and 3, 4 and 5, 2 and 9, 6 and 8, 7 and 10.

Allotetraploids are generally described in terms of separate genomes, designated A and B, in which corresponding chromosomes (1A, 1B; 2A, 2B; etc.) are those of similar phylogenetic derivation, hence those with the greatest degree of homology. Huskins (1931) suggested that such chromosomes "might be referred to as *homoeologous* chromosomes, signifying similarity but not identity" as in the case of *homologous*. Because of the confusion occasioned by the similarity of the two words, we have discarded *homoeologous* in favor of *analogous* to designate phylogenetically similar chromosomes.

The above indications of analogies among the ten haploid chromosomes of species of the subg. *Sorghum* are compatible with cytological studies which have been made on haploid plants of *S. vulgare*, including a haploid of line 'Experimental 3'. All investigators agree that occasional bivalents are found at late diakinesis and metaphase I of meiosis in pollen mother cells (Brown, 1943; Kidd, 1952; Endrizzi & Morgan, 1955; Sanders & Franzke, 1963). Rare associations of three or four have also been reported (Kidd, 1952; Sanders & Franzke, 1963). Endrizzi and Morgan (1955) considered that a reciprocal translocation and other abnormalities in progeny plants obtained by pollinating a haploid with a diploid of the same line resulted from crossing over in duplicate segments of partially homologous chromosomes, thereby providing evidence for the reality of pairing observed between chromosomes of the haploid.

Preliminary observations of pachytene chromosomes of sorghum haploids suggest that there are more paired than unpaired regions (Brown, 1943; Sanders & Franzke, 1963), and in haploid 'Experimental 3', cells at early diakinesis generally showed a multiple association including 5.5 chromosomes on the average. In some cells, all ten chromosomes were associated in a single complex in which most chromosomes were associated with at least two other chromosomes, some with more. The configuration seen in an individual cell appeared to depend on where separations had occurred in the complex. Cells at mid-diakinesis showed an average of 4.3 univalents and 2.1 bivalents with the remainder still in multiple associations. The large number of bivalents at this stage, in some cells four and very rarely five, suggests that some of the associations in the complex are more persistent than others and may indicate analogous chromosomes. The

occasional associations seen at late diakinesis and metaphase I may result from crossovers between like segments in analogous chromosomes, and the univalents may result from lack of crossovers due to the complexity of pairing relationships rather than from lack of pairing (Sanders & Franzke, 1963). These relationships between the chromosomes of an 'Experimental 3' haploid suggest that analogous chromosomes may differ by more than one translocation and agree with the interpretation suggested above for the work of Venkateswarlu and Reddi.

PAIRING OF ANALOGUES AND BIVALENT PAIRING MECHANISM. Analogous chromosomes in *Sorghum vulgare* would have to be sufficiently alike to pair under certain circumstances, and control of chromosome pairing would have to include a mechanism which promotes bivalent and limits multivalent pairing at the diploid level. These two assumptions are considered jointly because of interrelationships between them. Pairing of analogues is referred to by Waddington (1939, Chap. 2) and Stebbins (1947) as *heterogenetic association*. It is contrasted with *homogenetic association* (pairing of homologous chromosomes from the same genome), and is defined by Waddington as pairing between "homologous" chromosomes from different genomes when there is a considerable difference between them.

The most direct evidence for pairing of analogous chromosomes in *Sorghum* is furnished by the data on haploid plants cited above. Evidence that normal pairing is under genetic control is furnished by cases in which gene mutations have resulted in abnormalities. A spontaneous asynaptic mutation in 'Experimental 3' appeared to be a Mendelian recessive in relation to the normal (Ross *et al.*, 1960), and Huskins and Smith (1934) found an asynaptic strain of 'Dakota Amber Sorgo'. Examination of pairing relationships in sorghums with different chromosome complements gives some indication that a bivalent pairing mechanism may operate at certain chromosome levels in *Sorghum* and suppress formation of multivalents in some instances where they would otherwise be expected.

The degree of pairing in *Sorghum* would appear to depend on some sort of genetic balance which shifts with the genomes present. No evidence has appeared for localized control as in hexaploid *Triticum aestivum* where the presence of the long arm of chromosome V maintains bivalent pairing apparently without regard for the rest of the genotype (Riley *et al.*, 1960). However, there are correspondences between certain wheat plants lacking chromosome V and certain sorghum plants. In both species, pairing between analogous chromosomes occurs in haploids (Riley *et al.*, 1960; Sanders & Franzke, 1963) and in hybrids between species with different numbers of genomes, i.e., *T. aestivum* ($2n = 42$) $\times$ *Secale cereale* L. ($2n = 14$) (Riley *et al.*, 1960), and *Sorghum vulgare* ($2n = 20$) $\times$ *S. halepense* (L.) Pers. ($2n = 40$) (Hadley, 1953; Endrizzi, 1957). Some multivalent pairing occurs in *S. halepense*, but not enough to account for the degree of multivalent pairing in the hybrid (Celarier, 1958b). Addition of

chromosome V to the wheat plants eliminates analogous pairing, but controlling factors in *Sorghum* are not known.

Cytological studies of extra-chromosomal types at the diploid level in *Sorghum vulgare* have shown that the extra chromosomes occur more frequently as univalents than in multivalent configurations. For a trisomic derivative from a haploid, Endrizzi and Morgan (1955) reported 53 pollen mother cells with ten bivalents and a univalent, 19 with nine bivalents and a trivalent, and one with nine bivalents and three univalents. Extra-chromosomal types studied by Price and Ross (1957) were derived from an unknown triploid which they believed to be *S. vulgare* rather than a species hybrid. In 17 singly trisomic plants, ten bivalents and a univalent were found more frequently than nine bivalents and a trivalent at diakinesis and metaphase I; in three plants with two extra chromosomes, ten bivalents and two univalents were usually found; and in a fourth plant with two extra chromosomes, eleven bivalents were usually found.

Studies of multiple trisomics with from one to seven extra chromosomes in *Clarkia unguiculata* ($2n = 18$) showed an increase in trivalent formation with an increase in extra chromosomes. Trivalents per cell per extra chromosome were 0.43 for $2n + 1$, 0.46 for $2n + 3$, 0.56 for $2n + 4$, and 0.61 for $3n$ (Vasek, 1963). These results suggest that multivalent pairing may be influenced by a genetic balance which, in this case, shifts trivalent formation toward the frequency found in triploids when four or more extra chromosomes are present. Similar studies are needed in *Sorghum*.

Sorghums with a complement of 30 chromosomes have shown a high degree of trivalent formation whether $3n$ *S. vulgare* (Kidd, 1952), an unknown triploid (Price & Ross, 1957), or a hybrid between *S. vulgare* and either *S. halepense* or *S. almum* Parodi ($2n = 40$) (Hadley, 1953; Endrizzi, 1957). The number of trivalents per cell was highest for *S. vulgare* and *S. vulgare* × *S. almum* (from 8.2 to 8.5), and lowest for *S. vulgare* × *S. halepense* (from 5.7 to 5.8). Trivalent formation was consistently higher than the sum of trivalents and quadrivalents in corresponding 40-chromosome plants. Multivalent formation appears to be distinctly favored at the 30-chromosome level.

Cytological studies on tetraploids of *Sorghum vulgare* have shown a majority of the chromosomes associated as bivalents. In tetraploid *S. vulgare* var. *caffrorum* (Retz.) Hubb. & Rehd. (as var. *hegari* without authority, Chin, 1946), only three quadrivalents occurred per cell on the average and trivalents and univalents were rare. (Even the octoploid of the same variety had almost half the chromosomes in bivalent configurations.) In a tetraploid 'Experimental 3' plant, means of 3.3 quadrivalents, 0.04 trivalents, 12.8 bivalents and 1.2 univalents per cell were found (Sanders & Franzke, 1962b). Although multivalents are present uniformly in sorghum tetraploids, their formation is distinctly lower than in triploids, in spite of the increase in the number of homologous chromosomes.

These cytological data suggest several generalities concerning pairing relationships in *Sorghum* which need to be investigated. (1) Pairing of

analogous chromosomes may be limited chiefly to cases where a homologue is missing. Such a phenomenon could be designated as preferential pairing, the term used by Endrizzi (1957) to describe pairing relationships in *S. halepense*. (2) The tendency for bivalent pairing may be positively correlated with the presence of an even number of genomes, i.e., diploids and tetraploids. Configurations in *S. vulgare* haploids would have little bearing on bivalent or multivalent pairing as multiple associations appear to result from translocation differences between analogues. However, in triploids, trivalents are the rule. These relationships suggest that the bivalent pairing mechanism may be associated with genetic balance in some way. (3) Chromosome associations still present at diakinesis and metaphase I probably indicate only paired regions where crossovers occurred. Actual pairing relationships need to be studied at pachytene and diplotene.

Patterns of Chromosome Substitution

Based on the above assumptions, different patterns of chromosome substitution may be considered, and their results predicted.

INDIVIDUAL CHROMOSOMES. If a single chromosome were lost and replaced by its analogue, the chromosome number would be unchanged, and, with analogous pairing and a tendency for bivalent formation, ten bivalents would be seen at diakinesis and metaphase I. Such a plant could be either phenotypically mutant or unchanged depending on the shift in genetic balance effected by the chromosomes involved. The self-progeny would segregate to a greater or lesser degree in direct proportion to the amount of crossing over which occurred between the paired analogous chromosomes. If more than one individual chromosome were lost and replaced by its analogue, the chances of the plant being phenotypically mutant would be increased, and the degree of segregation in the self-progeny would be increased. Such plants would be diploid nontrue-breeding mutants and would segregate for many characters.

PAIRS OF CHROMOSOMES. If a pair of homologous chromosomes were lost and replaced by their analogues, the chromosome number would be unchanged, and ten bivalents would be seen at diakinesis and metaphase I. Such a plant would be phenotypically mutant and would produce a uniform self-progeny of the same mutant type. Replacement of more than one pair of homologous chromosomes by their analogues would increase the number of such types which could be obtained. Such plants would be diploid true-breeding mutants changed for fixed complexes of characters.

INDIVIDUAL AND PAIRED CHROMOSOMES. If both single chromosomes and pairs of homologous chromosomes were lost and replaced by their respective analogues, the chromosome number would be unchanged, and ten bivalents would be seen at diakinesis and metaphase I. Such a plant would

be phenotypically mutant and would produce a self-progeny which would be uniform for some characters present in the parent mutant and segregate for others. Substitution of different numbers of individual chromosomes and pairs of chromosomes would produce intermediate diploid mutants ranging from those which would produce self-progenies primarily segregating, to those which would produce self-progenies uniform for many mutant characters.

EVALUATION OF THE HYPOTHESIS

Relevant Effects of Colchicine

That chromosomes may be lost and gained other than as entire genomes in cells of colchicine-treated tissues is indicated by the aneuploid types which have been obtained following colchicine treatment. In addition to polyploid types (some with aneuploid numbers) obtained from colchicine-treated *Datura* seeds, Bergner, Avery, and Blakeslee (1940) found $2n - 1$ plants at about 70 times the rate, and $2n + 1$ plants at about three times the rate that they were obtained from untreated seeds. Smith (1943) obtained aneuploids following colchicine treatment of two *Nicotiana* species and their F_1 hybrids. Both at diploid and polyploid levels, more of these plants resulted from chromosome deficiencies than from additions. One plant with the actual tetraploid number of 36 was off-type and appeared to have lost some chromosomes and gained others. The effects of colchicine on dividing cells can account for such phenomena.

ARRESTED MITOSES. The primary observable cytological effects of colchicine result from the inactivation of the spindle mechanism in dividing cells (Levan, 1938; Eigsti & Dustin, 1955, Chap. 2). Mitoses are interrupted at metaphase, and, after a delay, chromosome divisions may occur without nuclear or cell division so that doubled or polyploid numbers of chromosomes may be built up within a single nucleus and cell. During recovery, processes which take place in dividing cells frequently resume more or less out-of-phase with one another and result in abnormalities other than polyploid nuclei. Arrested metaphases assume various patterns from a single clump with centromeres congregated at the center and chromosome arms extended, to an "exploded" type with chromosomes scattered throughout the cell. Multiple clumps and multipolar spindles are common also so that, when followed by subdivision of the cell, polyploid chromosome groups may be reduced to smaller ones (Eigsti & Dustin, 1955, Chap. 2).

Delay in the division of the centromere accompanies inactivation of the spindle and results in characteristic "c-pairs" in which the daughter chromosomes remain attached only at the centromeres (Levan, 1938). After division of the centromeres, daughter chromosomes continue to lie parallel to one another and may remain so through several chromosome divisions so that an accumulation of the same chromosome is built up in

one position. Division of the centromeres is not simultaneous for all the "c-pairs" in one nucleus. There appears to be ample opportunity for two daughter chromosomes to act either as a unit or as two individuals during the irregularities of "c-mitoses" thereby making substitution of chromosomes by homologous pairs as plausible as by single chromosomes. True-breeding, nontrue-breeding and intermediate mutants would all be expected.

The suggestion of Bergner *et al.* (1940), that colchicine-induced delay of mitosis may exaggerate irregularities such as lagging and nondisjunction which in nature occur occasionally, should also be considered.

REDUCTION OF CHROMOSOMES IN SOMATIC TISSUES. As indicated above, multiple clumps and multipolar spindles in cells undergoing "c-mitoses" provide mechanisms whereby chromosome numbers may be reduced as well as duplicated during colchicine treatment.

Reduction of chromosomes in somatic tissues and its concomitant effects need further investigation. Isolated reports of chromosome reduction divisions and of haploid cells in somatic tissues led to a study of the phenomenon by Huskins (1948) and his associates. Colchicine was found to induce reductional groupings of chromosomes in root tips of *Tradescantia* and *Allium* (Allen *et al.*, 1950), and of *Sorghum* including line 'Experimental 3' (Atkinson *et al.*, 1957). The concept of somatic reduction has been part of explanations offered for the origin of colchicine-induced mutants from the beginning (Franzke & Ross, 1952). Although haploid plants have been obtained from colchicine-treated diploid seedlings, they have been extremely rare, if anything less frequent than from untreated seedlings. In one progeny-test plot, haploids occurred at the rate of approximately one per 1156 diploid plants. They were about seven times more frequent in progenies of untreated than of treated parents; the latter progenies were untreated plants a generation removed from colchicine treatment. On the other hand, following colchicine treatment of tetraploid 'Experimental 3' seedlings, four out of nine surviving plants were diploid and mutant (Sanders & Franzke, 1962b). A fifth plant was mixoploid and mutant. Phenotypic examination of 42 untreated plants from the same seed source and cytological examination of 37 of these indicated that all were still tetraploid and apparently unchanged. The diploid mutants obtained from tetraploids demonstrate that cells derived from reduction divisions in colchicine-treated tissues may survive and function in the production of new shoot apices. Reduction appears to take place more readily following treatment of tetraploids than of diploids, suggesting that reduction may occur more frequently in polyploid cells than at lower levels.

It is suggested that duplication provides the extra chromosomes necessary for substitution and, perhaps, an unbalance which favors reduction in somatic cells, and that reduction effects the return to the diploid level and the simultaneous redistribution of the chromosomes. Both loss and gain of chromosomes are consistent with these known effects of colchicine.

Substitution of analogous chromosomes in colchicine-induced mutants in *Sorghum* could be attributed to selection among the cells of the treated growing point which allows only viable and preferably vigorous chromosome combinations to continue to reproduce themselves and organize a meristem capable of giving rise to a mutant plant. Observations of Levan (1938) are in accord with such an assumption. He noted, in root tips of *Allium*, that diploid cells are favored over polyploid cells during recovery after colchicine treatment and are usually more numerous near the apex.

Relevant Information on Colchicine-induced Mutants

The present explanation and the information that has been assembled on colchicine-induced mutants are generally in agreement. Several cases, however, remain controversial.

SIMILARITIES BETWEEN TRUE-BREEDING AND NONTRUE-BREEDING MUTANTS. There is no distinct separation between true-breeding and nontrue-breeding mutants since intermediate types appear which are true-breeding for some characters but not for others. All types may arise under the same conditions and in conjunction with one another (Sanders *et al.*, 1962). Such similarities suggest that all the diploid colchicine-induced mutants arise as variations of a single phenomenon. The present explanation accounts for all of the mutants as originating from different patterns of chromosome substitution.

"REACTOR" AND "NONREACTOR" LINES. That sorghum lines differ in their response to colchicine has been demonstrated in a comparison between the two lines 'Experimental 3' and 'Norghum' (Atkinson *et al.*, 1957). Lines such as 'Experimental 3' that produce a relatively high proportion of obvious diploid mutants following treatment have been termed "reactors," whereas those which produce only polyploids and minor variations have been termed "nonreactors." According to the present explanation, a line could give rise to such mutants if its chromosome complement included analogous pairs which could be substituted without impairing plant viability, and if the analogues were sufficiently different that substitution of one or both members of the pair resulted in obvious phenotypic changes. Such lines would have the fixed heterozygosity postulated by Huskins and Smith (1934) for sorghums with $2n = 20$ and would be "reactors." On the other hand, if a line had analogous pairs which could not be substituted without impairing plant viability, or if the analogues were not sufficiently different for substitution to result in obvious phenotypic changes, or if four homologues were present instead of two pairs of analogues in the five basic chromosome units, a line could not give rise to composite mutants by chromosome substitution and would be a "nonreactor." The many different types of sorghums have been so intermingled by hybridization that possible combinations of chromosomes from different sources would appear to be exceedingly large. Further, if pairing of

analogues occurs in certain hybrids and is accompanied by crossing over, new chromosomes unlike any of the originals would have been formed.

MUTANT INTERRELATIONSHIPS. Following colchicine treatment of one of the three Class I mutant lines shown to be genotypically as well as phenotypically alike (Chen *et al.*, 1961), four mutants were obtained which belong to three other classes of true-breeding mutants derived directly from 'Experimental 3' (Franzke & Sanders, in press). Also two true-breeding plants of the same type in an F_1 self-progeny from a nontrue-breeding mutant belong to a class derived directly from 'Experimental 3'. These results are consistent with the present explanation since a true-breeding or nontrue-breeding mutant derived directly from 'Experimental 3' could give rise to another true-breeding mutant type if chromosome substitutions which had taken place in the formation of the former did not preclude the substitutions necessary for the formation of the latter. If crossing over occurred between paired analogues in a nontrue-breeding mutant, chromosomes unlike any of the originals would be formed, and the probability of the self-progeny including types also derived directly from 'Experimental 3' would be decreased.

SEGREGATING PROGENIES FROM PHENOTYPICALLY UNCHANGED PLANTS. All true-breeding mutants and the majority of nontrue-breeding mutants have been recognized by phenotypic changes in the treated plants. However, some nontrue-breeding mutants have not been recognized until their self-progenies were grown. In some of these progenies there has appeared to be less segregation than in segregating progenies of recognized mutants (Sanders *et al.*, 1959), but this has not always been the case, i.e., mutant E (Sanders *et al.*, 1962). According to the present explanation, apparently unchanged treated plants could produce segregating self-progenies if only substitutions of individual chromosomes had occurred so that at least one of each of the ten haploid 'Experimental 3' chromosomes were still present, and if none of the substitutions shifted the gene balance sufficiently to be detected phenotypically.

APPARENT MUTATIONS OF SINGLE LOCI FROM DOMINANT TO RECESSIVE ALLELES. Genetic analyses of both true-breeding and nontrue-breeding mutants have provided instances of apparent single-gene changes, homozygous in the former and heterozygous in the latter (Foster *et al.*, 1961; Sanders *et al.*, 1962). Mendelian ratios for several qualitative characters were obtained in F_2 populations from crosses between true-breeding mutants and the parent line 'Experimental 3', and in first generation self-progenies of nontrue-breeding mutants. Except where the mutants were intermediate, having both nontrue-breeding and true-breeding mutant characters, nontrue-breeding mutants behaved genetically much like F_1 hybrids between true-breeding mutants and 'Experimental 3'. According to the present explanation, strictly nontrue-breeding mutants would be equivalent to such F_1 plants.

If 'Experimental 3' is a fixed heterozygote for many genes as the result of an allopolyploid origin at the same time it is a true-breeding line, then the dominant alleles for these genes would be present on both homologues of one chromosome pair and the recessive alleles would be present on both members of the analogous pair. If the pair carrying the dominant alleles were replaced by an additional pair carrying the recessive alleles, the recessive phenotype would appear in the mutant and all its progeny. If such a mutant were crossed with 'Experimental 3', the F_1 would contain three homologous chromosomes carrying the recessive allele and one analogue with the dominant allele, and would exhibit the dominant phenotype. With analogous and bivalent pairing, a 3:1 ratio for dominant: recessive phenotype would be obtained in the F_2 population. If an individual chromosome of 'Experimental 3' carrying a dominant allele were replaced by an additional chromosome carrying the recessive allele, the nontrue-breeding mutant would be genetically equivalent to the above F_1 plant for that particular unit of four chromosomes. It would seem that apparent mutations from dominant to recessive alleles may result from chromosome substitution rather than from point mutation.

COMPLEX DIPLOID MUTANTS NOT INDUCED BY COLCHICINE. Diploid mutants have arisen without colchicine treatment in self-progenies both of unstable 'Experimental 3' polyploids (Franzke *et al.*, 1962) and of 'Experimental 3' plants homozygous for an asynaptic gene (unpublished). These findings are in agreement with the present explanation since mutants should result from any interruptions of normal chromosome behavior which could result in new combinations. Multivalent pairing in a polyploid and lack of normal pairing in an asynaptic plant would result in gametes containing chromosome groups other than the normal complement. Diploid mutants could result from viable combinations either in $2n$ egg cells which developed parthenogenetically, or in haploid egg cells or pollen grains which effected fertilization. As would be expected, the three mutants from the asynaptic line were nontrue-breeding. The two diploid mutants found in polyploid self-progenies were true-breeding. Yet progeny plants in both groups showed an excess of univalents during meiosis I.

CHROMOSOME IRREGULARITIES IN NONTRUE-BREEDING MUTANT PROGENIES. Although irregularities of chromosome behavior have not thus far been found in true-breeding or nontrue-breeding mutants obtained from colchicine-treated diploid seedlings, some abnormalities were found in plants from nontrue-breeding mutant progenies (Sanders & Franzke, 1962a). The principal abnormality was an increase over untreated plants in the number of univalents at diakinesis and metaphase I in pollen mother cells. Rare trivalents and quadrivalents were also found, but since several investigators have reported occasional multivalents in various diploid sorghum lines (Huskins & Smith, 1934; Chin, 1946; Hadley, 1953), these cannot necessarily be attributed to the mutant condition. The increase in univalents in these plants is in agreement with the present explanation

since the pairing of analogous chromosomes in nontrue-breeding mutants would be expected to result in some chromosome irregularities in the progeny plants, especially if crossing over had occurred and interchanged segments of the analogues. Irregularities in pairing relationships should be sought in nontrue-breeding mutants, F_1 hybrids between true-breeding mutants and their parent lines, and plants from nontrue-breeding self-progenies and from F_2 populations. Crossovers between analogues would be equivalent to translocations between these chromosomes, but the usual translocation configurations would not be expected if there is a bivalent pairing mechanism.

APPARENT MUTATIONS FROM RECESSIVE TO DOMINANT ALLELES. 'Experimental 3' exhibits the dominant phenotypes of the three observed characters which might be attributed to single-gene differences. These are red as opposed to green seedling-base, dry as opposed to juicy stalk and midrib, and awnless as opposed to awned spikelets (Quinby & Martin, 1954). A Class I mutant line which exhibits the recessive phenotypes for all three characters, when treated with colchicine, gave rise to mutant lines belonging to three other classes which included at least one apparent mutation from recessive to dominant for each of the characters (Franzke & Sanders, in press). According to the present explanation, the Class I mutant would have resulted from the replacement of pairs of homologous chromosomes carrying the dominant alleles by their analogues carrying the recessive alleles. No further substitution would be possible in these units of four chromosomes since they would now consist of four homologues, and a source for the return to the dominant phenotype is not evident.

However, sorghum genetics is not sufficiently understood to make a reliable interpretation of genotype on the basis of phenotypic observation alone. Similar phenotypes in sorghums have been found to result from different genotypes, alleles of certain genes have been found to have different phenotypes in different genetic constitutions, and gene interaction has been found to be common (Celarier, 1958a). Class I mutants have been analyzed genetically in relation to 'Experimental 3' and to each other (Chen *et al.*, 1961). It would be necessary to analyze mutant lines from the classes obtained both from Class I and from 'Experimental 3' in relation to both parental lines before any conclusions could be drawn.

In other species, characters identified with particular genes have been found to change phenotypically in chromosome mutants without the chromosome carrying the known gene being involved, i.e., in *Datura* (Blakeslee, 1922) and in tomato (Lesley, 1928). Rick and Barton (1954) noted little or no relationship between the phenotype of a trisomic in tomato and the genes known to be on the extra chromosome. Such results indicate the complexity of phenotypic effects brought about by chromosome changes and suggest that apparent changes from recessive to dominant phenotypes might come about through chromosome substitution.

CHROMOSOME PAIRING RELATIONSHIPS IN COLCHICINE-INDUCED DIPLOID MUTANTS. Because of the presence of ten bivalent chromosomes at diakinesis and metaphase I of meiosis in pollen mother cells of colchicine-induced diploid mutants and their F_1 hybrids with other sorghum lines, the present explanation depends on analogous pairing and on a mechanism for bivalent pairing in diploid sorghums with 20 chromosomes. As cited above, instances of analogous pairing in *Sorghum* are known. The bivalent pairing mechanism provides the greatest difficulty since, as presented earlier, multivalents are found in *Sorghum*: in tetraploids, especially in triploids, sometimes in trisomics at the diploid level although a bivalent and a univalent are more usual, and very occasionally in straight diploids where they would seem to involve analogous pairing. It is on the basis of the rarity of multivalents in diploids and their relative infrequency in trisomics that it is proposed that bivalent pairing may be the rule and multivalent pairing the exception at the 20-chromosome level. Following a polyploid origin, the sorghums with $2n = 20$ may have undergone "diploidization" which included the establishment of bivalent pairing even though the analogous chromosomes retain the ability to pair with one another, especially when uneven numbers of homologues are present. However, a clearcut understanding of the control of bivalent pairing, such as that worked out for hexaploid wheat, is needed.

IMPLICATIONS OF THE HYPOTHESIS FOR THEORETICAL AND APPLIED RESEARCH

If colchicine-induced diploid mutants in *Sorghum* result from substitution of analogous chromosomes, it should be possible to obtain the same results with other plants of allopolyploid derivation in which analogous chromosomes are sufficiently similar that they can be interchanged without an adverse effect on plant viability. If such mutants were to be easily recognizable, it would be necessary that the analogous chromosomes carry different alleles which would result in distinct phenotypes, and that the original line be true-breeding and preferably not too highly polyploid so that changes would not be obscured by the variation in the original population, or covered by the multiple effects of alleles of the same genes on other chromosomes. In *Linum usitatissimum* L., colchicine treatment of seedlings heterozygous for known genes resulted in one plant with branches homozygous for the gene markers (Dirks *et al.*, 1956). Homologous chromosomes carrying different alleles may have been substituted for one another. Since this species ($2n = 30$) is thought to be of polyploid and perhaps of hybrid origin (Ray, 1944), it might also be expected to produce diploid mutants following treatment of seedlings of true-breeding lines. Meanwhile, the results suggest that, in heterozygous plants not necessarily of allopolyploid origin, it may be possible to induce substitution of homologous chromosomes for one another by colchicine treatment, and hence to obtain homozygosity from heterozygosity without generations of inbreeding.

As stated earlier, there is some evidence that diploid mutants are obtained under conditions of colchicine treatment more stringent than those required to produce polyploids. Success in obtaining mutants depends on the skill of the researcher in insuring survival of treated seedlings under adverse circumstances. Environmental factors favorable for the production of mutants probably vary with the material, but if conditions necessary to obtain polyploids with colchicine are known, they could be taken as the starting point from which to develop more rigorous methods of treatment while still keeping the plants alive. Treated seedlings under favorable growing conditions appear to outgrow the effects of colchicine (Sanders *et al.*, 1959). It would seem necessary to delay growth long enough to allow the drug not only to act (duplication of chromosomes to produce polyploid cells), but also to react (subdivision of polyploid cells to produce cells with viable new diploid complements) while still keeping the material alive. To what extent mutants can be obtained by colchicine treatment of plants other than *Sorghum* needs to be determined.

In either a research or a breeding program, colchicine treatment would provide a method for obtaining recombinations of substitutable chromosomes, sometimes accompanied by homozygosity, within the genetic complement either of an established line or of a hybrid. The method has been used in the sorghum-breeding program at South Dakota State College, and new true-breeding lines have been obtained and released as agronomic varieties following treatment both of seedlings from true-breeding lines, i.e., 'Winner' (Class II, Franzke & Sanders, in press), and of hybrid seedlings, i.e., 'Dual' (Franzke, 1958). If substitution of individual chromosomes does occur, crossing over between paired segments of analogous chromosomes could result in recombination of parts of chromosomes as well as of whole chromosomes in the progeny.

If the hypothesis is correct, the method in use in the sorghum program is basically similar to the new approach to plant breeding described by Riley (1963) for wheat. New possibilities are opening up following the discovery that elimination of chromosome V removes the limitations imposed by bivalent pairing and permits recombinations of chromosomes and chromosome segments between genomes. He writes, "In wheat we now have the first example of a system by which recombination between different parental patterns can be modified. The impact of this notion on plant breeding as a whole has still to be determined, but it could be considerable." It may prove possible to obtain recombinations between different genomes in a plant by using colchicine to bring about chromosome substitutions in somatic tissues, thus bypassing established bivalent pairing mechanisms. Use of asynaptic plants to interchange chromosomes between genomes should also be investigated.

The degree of variability which might be obtained by using the method would depend on the genetic constitution of the original material. Treatment of a polyploid line which is a fixed heterozygote for many genes should provide a limited example of Stebbins' (1940) description of possibilities for evolution within a polyploid complex: "It can produce

endless new species, but these are all or nearly all new combinations of the same supply of genic material; they are new variations on an old theme."

SUMMARY

An hypothesis is presented that explains the diploid mutants which arise in certain lines of *Sorghum* following colchicine treatment of seedlings as resulting from the substitution of analogous chromosomes originally present in the genetic constitution of sorghums with $2n = 20$.

Nontrue-breeding mutants are formed if there are substitutions only of individual chromosomes; true-breeding mutants, if there are substitutions only of pairs of homologous chromosomes; intermediate mutants, if there are substitutions of both individual chromosomes and pairs.

These chromosome substitutions are not detected at diakinesis or metaphase I since no abnormal pairing configurations result. Pachytene and diplotene chromosomes in nontrue-breeding mutants and in F_1 hybrids involving true-breeding mutant lines need to be carefully examined for evidence of analogous pairing.

Sorghum and other species, particularly those of polyploid and hybrid origin, need to be investigated intensively in order to establish whether this hypothesis concerning colchicine-induced chromosome substitution is valid.

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